Pest Manag Sci **61**:75–84 (2005)
DOI: 10.1002/ps.949

Spatial and temporal distribution of imidacloprid and thiamethoxam in citrus and impact on *Homalodisca coagulata* populations

Steven J Castle,[1]* Frank J Byrne,[2] Jian L Bi[2] and Nick C Toscano[2]

[1]*USDA-ARS Western Cotton Laboratory, 4135 E Broadway Road, Phoenix, AZ 85040, USA*
[2]*Department of Entomology, University of California, Riverside, CA 92521, USA*

Abstract: Titers of two systemic neonicotinoid insecticides in citrus trees were measured in conjunction with conventional evaluations of their impact on glassy-winged sharpshooter (*Homalodisca coagulata* (Say); GWSS) populations. Xylem fluid samples were collected at regular intervals and from multiple locations within field-grown citrus trees to determine imidacloprid and thiamethoxam concentrations using commercial ELISA kits. Uptake profiles varied considerably with peak mean titers of imidacloprid occurring 6–8 weeks after application compared with 2 weeks for thiamethoxam. The persistence of each compound also varied as near-peak levels of imidacloprid were sustained for another 6–10 weeks before gradually declining. In contrast, thiamethoxam titers declined more rapidly after the initial peak, possibly reflecting an application rate only one-quarter of that used for imidacloprid. Within-tree distributions were more similar for the two compounds, with no significant effect due to height of the sample (upper or lower half) or to the quadrant location within the tree, with the exception of one quadrant in the thiamethoxam-treated trees. Substantial reductions in GWSS nymphs and adults were observed in imidacloprid-treated trees during the 2001 trial and were sustained for 4–5 months after treatment. Treatment effects on nymphs were not as well pronounced in the 2002 trial, when overall GWSS infestations were much reduced from the previous year. However, consistently lower adult infestations were still observed in 2002 for both treatments compared with untreated trees. Information on the spatial and temporal profiles in citrus trees was obtained for both compounds to complement field impact data and improve understanding of their pest management potential.

Keywords: imidacloprid; thiamethoxam; *Homalodisca coagulata*; systemic uptake; xylem fluid; ELISA; pest management

1 INTRODUCTION

The duration that an insecticide is active in a crop in terms of lethal and sub-lethal effects on insect populations is a critical concept in pest management that has been poorly developed. Information on the persistence of an insecticide is often broadly generalized and tailored for health and environmental concerns rather than for pest management purposes. Residue information on a product label is usually presented in the context of re-entry periods following insecticide applications instead of the rate that field control diminishes over time. Data on the persistence and activity of insecticide residues in crop canopies could improve the efficiency of insecticides used against crop pests. Application rates, the timing and spacing of applications, as well as the choice of applications could all be influenced by more reliable information on the persistence of insecticides in crops. Greater confidence in the fate of an application might help reduce 'insurance' treatments that pest managers are sometimes compelled to apply because of uncertainty about persistence in the crop and the level of control being exerted on a target population. However, the considerable costs of conducting residue tests may have precluded efforts to gather residue information that could improve understanding of an insecticide's activity period in a crop.

A recent example from California points to the need for improving information about when an insecticide is working effectively to suppress target populations. The epidemic of Pierce's disease in the vineyards of Temecula in Riverside County, California brought into focus the urgent need to control glassy-winged sharpshooter (*Homalodisca coagulata* (Say); GWSS) populations, especially around vineyards but also throughout the state in general. As a new species to California, GWSS was officially recognized from a specimen collected in Irvine, CA in 1989.[1]

* Correspondence to: Steven J Castle, USDA-ARS Western Cotton Laboratory, 4135 E Broadway Road, Phoenix, AZ 85040, USA
E-mail: scastle@wcrl.ars.usda.gov
(*Received 17 November 2003; revised version received 10 May 2004; accepted 2 July 2004*)
Published online 9 September 2004

Before long, however, establishment had occurred in agricultural and urban regions throughout southern California. Although there was ample awareness of the high populations of GWSS and their vectoring capability based on the oleander leaf scorch epidemic in southern California oleanders beginning in the early 1990s,[2] it was not until vineyards in Temecula began to die in mass[3,4] that the unfolding crisis in California's agriculture was recognized and acted upon at local, state and federal levels.

Beginning in the spring of 2000, a regional control project for GWSS in Temecula was initiated collaboratively by the California Department of Food and Agriculture (CDFA) and the University of California, Riverside with Federal funding provided by USDA-APHIS. Instituted as an abatement action, the area-wide program sought to apply effective treatments that would drastically reduce population densities of GWSS. It was widely recognized at this time that citrus served as a major source of GWSS in the Temecula vineyards[5] as well as host to the first generation of offspring produced each year. The abatement strategy in Temecula involved the systemic treatment of approximately 810 ha of citrus with imidacloprid (Admire®) applied through the irrigation systems equipped with mini-sprinklers. Upon completion of the applications, questions began to arise concerning the level of control associated with the imidacloprid treatments, especially in older, mature citrus trees. Few guidelines were available regarding the activity of imidacloprid in mature citrus against GWSS, or what pattern of uptake and spatio-temporal distribution within a tree might occur. Thus, little reassurance could be provided to growers and pest managers concerned with reducing GWSS populations and involved directly or indirectly with evaluating the abatement program. Consequently, a substantial portion of the area treated with imidacloprid was re-treated with chlorpyrifos (Lorsban®).

In order to develop IPM guidelines for managing GWSS in citrus, it became clear that groundwork information on the activity profile of imidacloprid in citrus would be required. Decision-making in pest management ideally should incorporate basic knowledge of an insecticide such as its mode of action, the nature of the exposure to the target pest, including its spatial and temporal dynamics, and the intrinsic susceptibility of the target pest to the chosen insecticide. For a systemic insecticide such as imidacloprid, this information is often less apparent than for a foliar contact insecticide, in part because of the longer period required for translocation throughout a plant compared with the immediate contact and exposure of a foliar-applied insecticide. Therefore, our goal in this study was to measure titers temporally and spatially of two systemic neonicotinoid insecticides, imidacloprid and thiamethoxam, and determine their impact on GWSS infestations in citrus.

2 MATERIALS AND METHODS

2.1 Study site

Experimental fieldwork was carried out at the University of California's Agricultural Operations in Riverside, California during 2001 and 2002. This research farm comprises over 500 ha, nearly half of which is planted to a wide assortment of mostly untreated citrus. Large populations of GWSS developed on the university farm during the 1990s, making it an ideal setting for testing the impact of imidacloprid and thiamethoxam in citrus.

In 2001, work was carried out in a block of 10 rows (6.4 m centers) split equally between 30+-year-old oranges (var Frost Valencia grafted on Troyer citrange) and lemons (var Lupe grafted on Cook) situated in the center of a 12-ha orchard. Two contiguous rows consisting of 18 trees each (6.1 m spacing) out of the five rows of orange trees were treated with imidacloprid for a total of 36 trees. This left two untreated rows on one side and a single untreated row on the other side that was contiguous with the first row of lemons that also remained untreated. The five rows of lemon trees were divided into halves by an access road. Imidacloprid was applied to 22 lemon trees in four consecutive rows in the top half, leaving 33 trees in the bottom half along with the first row of lemon trees untreated. Adjacent orange and lemon trees on all four sides of the study block remained untreated throughout the experiment.

During the following year, the study was conducted in oranges only in a block of 12 new rows adjacent to those used in 2001. Two access roads divided this block into three sections with the upper and lower sections treated with imidacloprid and thiamethoxam, respectively, leaving the middle section untreated. The treatments were applied to eight contiguous rows within their respective sections, leaving two rows on either side untreated. A total of 48 trees were treated with each compound, leaving 24 untreated trees within each treatment section to serve as controls. With the expanded study area in 2002, it was possible to establish a subset of 24 treated trees for sampling that was completely within a border of treated trees.

2.2 Insecticide applications

Applications of imidacloprid and thiamethoxam were made through the irrigation system equipped with two micro-emitter sprinklers per tree. Imidacloprid was applied both years as 240 g liter^{-1} SC (Admire 2) at the rate of 2.34 liter ha^{-1} (32 fl oz acre^{-1}), ie the top recommended rate. Thiamethoxam was applied the second year only as 240 g liter^{-1} SL (Platinum®) at the rate of 0.58 liter ha^{-1} (8 fl oz acre^{-1}). Application dates were 10 April in 2001 and 4 April in 2002. Trees in the test area were irrigated 1–2 days before the application to wet the soil throughout the root zone. The irrigation system was run again for approximately 1 h prior to injecting any material in order to wet the soil surface. Imidacloprid SC (164 ml) and thiamethoxam SL (41 ml) were placed in individual

18.9-liter stainless steel cylinders which were brought to full volume with water, pressurized to 0.70 kg cm^{-2} above the irrigation system's operating pressure with carbon dioxide cartridges, and then injected at a rate of approximately 0.3 liter min^{-1} into the 2.54-cm poly irrigation lines. A red dye (Mark-IT, Monterey Chemical Co, Monterey, CA, USA) was added to the contents of the cylinders to confirm that the materials were delivered to each tree in the designated rows, but also, on the basis of absence of color, to notify when each compound was no longer being emitted from the mini-sprinklers. Sprinklers were then allowed to run another 1–2 h after the injections to flush the lines clear of material and to help move the treatment materials through the leaf litter into the soil.

2.3 Sampling

2.3.1 Insects

Because the field experiments were conducted in mature citrus orchards with trees 7+ m in height, a sampling device was developed that would permit access to foliage at both upper and lower reaches of the trees. This device (bucket sampler) consisted of a rigid-sided plastic bucket (11.4 liter) firmly attached to a telescoping painter's extension pole (3.7 m). The bottom of the bucket was cut away and a large-volume funnel (vertical-sided at the top 5 cm) was riveted to the bottom sides of the bucket. The apex of the funnel was removed and replaced with a plastic jar lid that was permanently attached to the bottom of the funnel. This permitted the plastic jar (0.5 liter) to be rapidly removed from the bottom of the bucket assembly in order to empty sample catches and then easily replaced for the next sample. The rigid-sided plastic bucket was durable enough to be thrusted repeatedly into the thick and resistant citrus foliage, yet light enough to allow continuous handling even at higher locations in the tree canopies. The thrusting of the bucket sampler into the dense canopy caused GWSS adults and nymphs to be dislodged from their feeding positions on stem branches and fall into the bucket, through the funnel and into the collecting jar. A sample consisted of four to six rapid thrusts at five locations around each tree. The contents of the collecting jar were then emptied into pre-labeled ziplock bags before moving on to the next tree.

In 2001, sampling was conducted weekly from 23 April through 16 November. A total of 12 treated and 12 untreated orange trees were sampled each week. Because there was a total of 36 treated and 36 untreated orange trees, it was possible to alternate weekly among three sets of 12 trees in each treatment group. This three-week rotation helped to ensure that the local population was not diminished through repeated sampling. Similarly, three sets of seven lemon trees, treated and untreated, were alternated every three weeks for GWSS sampling. In 2002, sampling was conducted weekly between 4 April and 11 July, then every other week until 17 October. Two sets of 12 orange trees were alternately sampled every other week for GWSS adults and nymphs in both the imidacloprid and thiamethoxam treatment blocks. Weekly alternation between two sets of 12 untreated orange trees was also carried out on the same schedule as the treated trees in 2002. All samples from both years were held in their ziplock bags and stored in a freezer (−20 °C) upon return from the field. All nymphs and adults in each bag were counted to determine whether there were density differences between treated and untreated trees, and between imidacloprid- and thiamethoxam-treated trees.

2.3.2 Xylem fluid

A cohort of trees within each block of treated trees was randomly selected for xylem-fluid sampling at two-week intervals throughout each six-month evaluation period. Xylem fluid was collected from 12 orange trees and 7 lemon trees in 2001, and from two new sets of 12 orange trees in 2002 treated with either imidacloprid or thiamethoxam. Two branch terminals from each tree, one from the north side and the other from the south side, were sampled every two weeks for xylem fluid for a total of 24 branch terminals from orange trees to evaluate imidacloprid titers both years, and from an additional 24 branch terminals in 2002 to evaluate thiamethoxam titers; 14 branch terminals from lemon trees were sampled every two weeks to evaluate imidacloprid titers in 2001. Xylem fluid samples from untreated orange and lemon trees were also periodically sampled as a null check for the evaluation of imidacloprid and thiamethoxam titers in xylem fluid. In addition, pre-treatment xylem fluid samples of all trees in each treatment cohort were collected to establish the initial absence of either imidacloprid or thiamethoxam in the experimental trees.

The spatial distribution of imidacloprid was evaluated in 2001 by intensively sampling four orange trees on three different dates at approximately eight-week intervals beginning eight weeks post-treatment. In 2002, three orange trees each were evaluated for imidacloprid and thiamethoxam distributions on four different dates at approximately six-week intervals beginning six weeks post-treatment. Each intensively sampled tree was divided in the vertical plane into four cardinal quadrants and the horizontal plane into upper and lower halves to yield eight sections per tree. Xylem fluid was collected from three branch terminals per section for a total of 24 branch terminals per tree for each sample date.

Stainless steel pressure cylinders (Soil Moisture Corp, CA) were used to extract xylem fluid from branch terminals of all lemon and orange trees. Compressed air cylinders were transported to the field along with two pressure cylinder units to carry out xylem extraction operations on the tailgates of pickup trucks. Branch terminals were cut to a length of 30–40 cm and positioned with leaves and secondary branches inside the cylinder with the severed primary branch protruding at least 4 cm through the screw-clamp opening of the pressure cylinder cap. Prior to clamping within

the cap of the pressure cylinders, the severed ends of branch terminals were trimmed of their cambium layers so that only xylem protruded from the cylinder caps. The cylinders were pressurized to 25–30 kg cm^{-2} to force xylem fluid out of the branch terminal. A pipettor (1000 µl) was used to collect the xylem fluid as it exuded from the severed end of branch terminals. Xylem fluid was transferred to pre-labeled microcentrifuge tubes (1.5 ml) in a series of aspiration steps until at least 150 µl of fluid had been collected. Each tube was then capped and placed immediately into a tube rack sitting on a block of dry ice within an ice chest for rapid freezing of each xylem fluid sample, then permanently stored at −30 °C in a laboratory freezer. New pipette tips were used for each branch terminal. Xylem fluid was consistently collected early mornings the day after irrigation so that soil moisture would be approximately the same for each sampling date.

2.4 Chemical quantification

Concentrations of imidacloprid and thiamethoxam within xylem fluid samples were determined using a competitive ELISA technique in which insecticide present within xylem fluid extracts competed with horseradish-peroxidase-labeled insecticide for a limited number of antibody binding sites contained within the wells of polystyrene microplates. The levels of bound conjugate were determined colorimetrically and were inversely proportional to the levels of insecticide present in the xylem fluid. Manufacturer's recommendations (EnviroLogix Inc, Portland, ME, USA, at www.envirologix.com, imidacloprid plate kit, cat # 006; Beacon Analytical Systems Inc, Portland, ME, USA, at www.beaconkits.com, thiamethoxam plate kit, cat # CPP-022) were followed for both insecticides, except that samples were mixed with the conjugate before addition to the immuno-assay plate. A series of standard concentrations were included in each ELISA test and used to generate a standard curve by which the levels of insecticide in samples could be accurately quantified. A Spectramax microplate reader and Soft-Max Pro curve-fitting software (Molecular Devices Inc, Sunnyvale, CA, USA, at www.moleculardevices.com) were used to measure end-point absorbance at 450 nm of each xylem fluid sample and determine the level of insecticide according to the standard curve, respectively. Preliminary experiments established that the binding characteristics of the standards supplied with the kits were comparable with a series of standards prepared directly in xylem, thereby indicating the absence of interfering matrix effects. The linear range of detection was 0.2–6 µg liter^{-1} for imidacloprid and 0.05–2 µg liter^{-1} for thiamethoxam. When readings measured above these limits, samples were diluted with distilled water and re-tested.

2.4 Data analyses

Analysis of variance (ANOVA) was used to determine whether there were significant differences in GWSS densities among treated and untreated trees or in the distribution of imidacloprid or thiamethoxam within or among trees. To account for possible correlation of measurements across time, a repeated measures multivariate analysis of variance (MANOVA) was used to evaluate GWSS densities based on a sphericity test that indicated in all cases the multivariate F-test to be more appropriate than a univariate F-test. Both between-subject and within-subject effects were examined for significance of F tests, and in the 2002 data, where three treatments (imidacloprid, thiamethoxam and untreated control) were considered, contrasts were used to further assess significant effects. All F statistics are presented as an adjusted univariate F statistic following multivariate analysis. A simple nested model ANOVA was used to examine whether significant differences in imidacloprid or thiamethoxam titers occurred in orange trees.

3 RESULTS

3.1 Temporal distribution

The temporal pattern of imidacloprid distribution in orange trees both years and in lemon trees in 2001 (Fig 1) was consistent, even though seasonal mean titers varied. For example, higher mean titers of imidacloprid were measured in 2002 oranges (up to week 26 post-treatment) compared with 2001, and both were higher than the 2001 lemons. The rate at which imidacloprid was taken up by orange trees in 2002 was more similar to the rate profile observed for lemons, with peak mean titers in both attained at six weeks post-application. In contrast, eight weeks were required in 2001 before mean titers >10 µg liter^{-1} were attained in oranges. Titers of imidacloprid then remained above or near 10 µg liter^{-1} in oranges through week 24 each year before dropping permanently below 5 µg liter^{-1} in week 26. A similar persistence of imidacloprid in xylem fluid was observed in lemon trees in 2001, albeit at levels lower than in oranges (Fig 1).

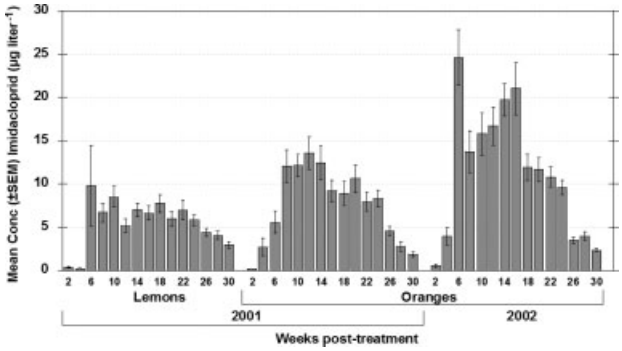

Figure 1. Temporal profiles of mean (±SEM) titers of imidacloprid in lemons and oranges in 2001 and in oranges again in 2002. The application date in 2001 was 10 April and in 2002 it was 4 April. Sample size for each date was $n = 8$ for lemons and $n = 12$ for oranges.

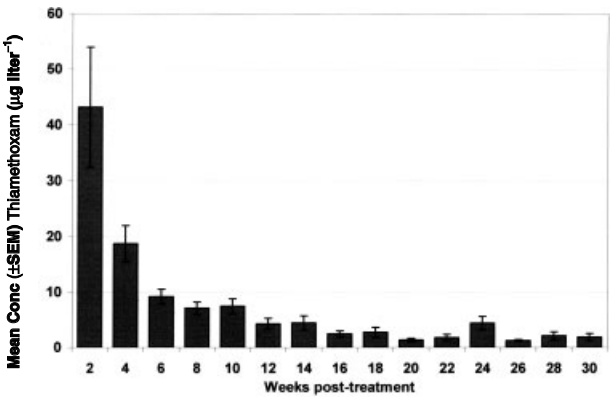

Figure 2. Temporal profile of mean (±SEM) titers of thiamethoxam in oranges in 2002. Sample size for each date was $n = 12$.

The temporal profile of thiamethoxam distribution in orange trees in 2002 (Fig 2) was substantially different from that observed for imidacloprid in orange or lemon trees in either year. The peak mean titer of thiamethoxam was observed two weeks after treatment application compared with a minimum six-week period required for peak mean titers of imidacloprid as observed in both lemons and oranges. By six weeks post-treatment, mean titers of thiamethoxam had dropped below $10\,\mu g\,liter^{-1}$ and continued to decline progressively through the remainder of the monitoring period. However, the highest mean titer of thiamethoxam at two weeks post-treatment (43.2 (±10.8) $\mu g\,liter^{-1}$) was nearly 75% greater than the highest mean titer of imidacloprid (24.7 (±3.2) $\mu g\,liter^{-1}$) observed in either year despite an application rate that was only one-quarter of that for imidacloprid.

3.2 Spatial distribution

Within-tree distribution of imidacloprid was remarkably consistent both years in orange trees. The profiles of imidacloprid titers within each of the eight sections showed little difference between lower and upper sections of the trees or among the four quadrants on all three sample dates in 2001 (Fig 3a) and all four sample dates in 2002 (Fig 3b). No significant differences in imidacloprid titers were observed in 2001 between the lower and upper halves of the trees ($F_{1,268} = 1.63$, $P = 0.203$) or among the four quadrants nested within height [quadrant(height) $F_{6,268} = 1.28$, $P = 0.266$]. The same was found in 2002 with no significant differences between lower and upper halves ($F_{1,268} = 1.10$, $P = 0.30$) or among the four quadrants nested within height [quadrant(height) $F_{6,268} = 1.93$, $P = 0.08$]. However, highly significant differences were observed among the four trees that were intensively sampled in 2001 ($F_{3,268} = 12.96$, $P < 0.0001$) and among the three intensively sampled trees in 2002 ($F_{2,268} = 24.33$, $P < 0.0001$). As might be expected, sample date also proved to be a source of significant variation both years. In 2001, differences among the three sample dates were highly significant ($F_{2,268} = 27.19$, $P < 0.0001$) as they were again among four dates in 2002 ($F_{3,268} = 59.16$, $P < 0.0001$). Highly significant interactions among trees and sample dates [tree × sample date] were observed in 2001 ($F_{6,268} = 15.14$, $P < 0.0001$) and again in 2002 ($F_{6,268} = 3.32$, $P = 0.0036$).

At the time of the first sample to measure the spatial distribution of thiamethoxam, titers of thiamethoxam had already declined considerably from their peak levels. Thus, the mean and range of thiamethoxam titers (Fig 4) registered much lower than those for imidacloprid (Fig 3). Moreover, significant within-tree variation was registered in the model effect quadrant[height] ($F_{6,275} = 2.86$, $P = 0.0102$) in contrast to the more uniform within-tree distribution of imidacloprid. Part of this difference in the within-tree distributions between the two compounds may be due to the different temporal profiles of each one. There was, however, no significant variation in the height component ($F_{1,275} = 0.04$, $P = 0.84$) of the within-tree distribution of thiamethoxam. Other similarities to imidacloprid included highly significant differences among sample date ($F_{1,275} = 23.43$, $P < 0.0001$) and trees ($F_{2,275} = 21$, $P < 0.0001$), and significant variation in the interaction term [tree × sample date] ($F_{2,275} = 4.71$, $P = 0.0098$).

3.3 Impact on glassy-winged sharpshooters

Clear differences in GWSS densities between imidacloprid-treated and untreated trees were observed in 2001 when populations were much larger than in 2002. For the first four weeks following treatment, nymphal densities were high in both treated and untreated trees (Fig 5a). At week 6 post-treatment, a sharp decline in nymphal densities occurred in the imidacloprid-treated oranges coinciding with mean titers of imidacloprid surpassing $5\,\mu g\,liter^{-1}$. As mean titers of imidacloprid continued to rise, mean nymphal densities fell to 4.4 (±1.4) by week 8 compared with 30.49 (±4.4) in the untreated control. Attrition of nymphs through natural mortality, emigration and, beginning at week 9, emergence to the adult stage, also contributed to declining nymphal densities as suggested by a similar trend in the untreated control. However, mean nymphal densities remained between 30 and 40 through week 10 in the untreated trees while mean densities dropped below 2 at week 10 in the imidacloprid-treated orange trees (Fig 5a). Season-long differences between treated and untreated nymphal densities were highly significant ($F_{1,22} = 28.1$; $P < 0.0001$) based on a repeated measures MANOVA.

Adults were extremely scarce through the first eight weeks of GWSS sampling, on many dates limited to just one caught per 24 trees (treated and untreated) sampled (Fig 5b). With maturation of the first nymphs and emergence to adults, numbers of adults rapidly increased in both treated and untreated oranges between weeks 9 and 12 (15 June–6 July). The high numbers in both treatments was an indication of the frenzy of flight activity that was apparent in the orchard

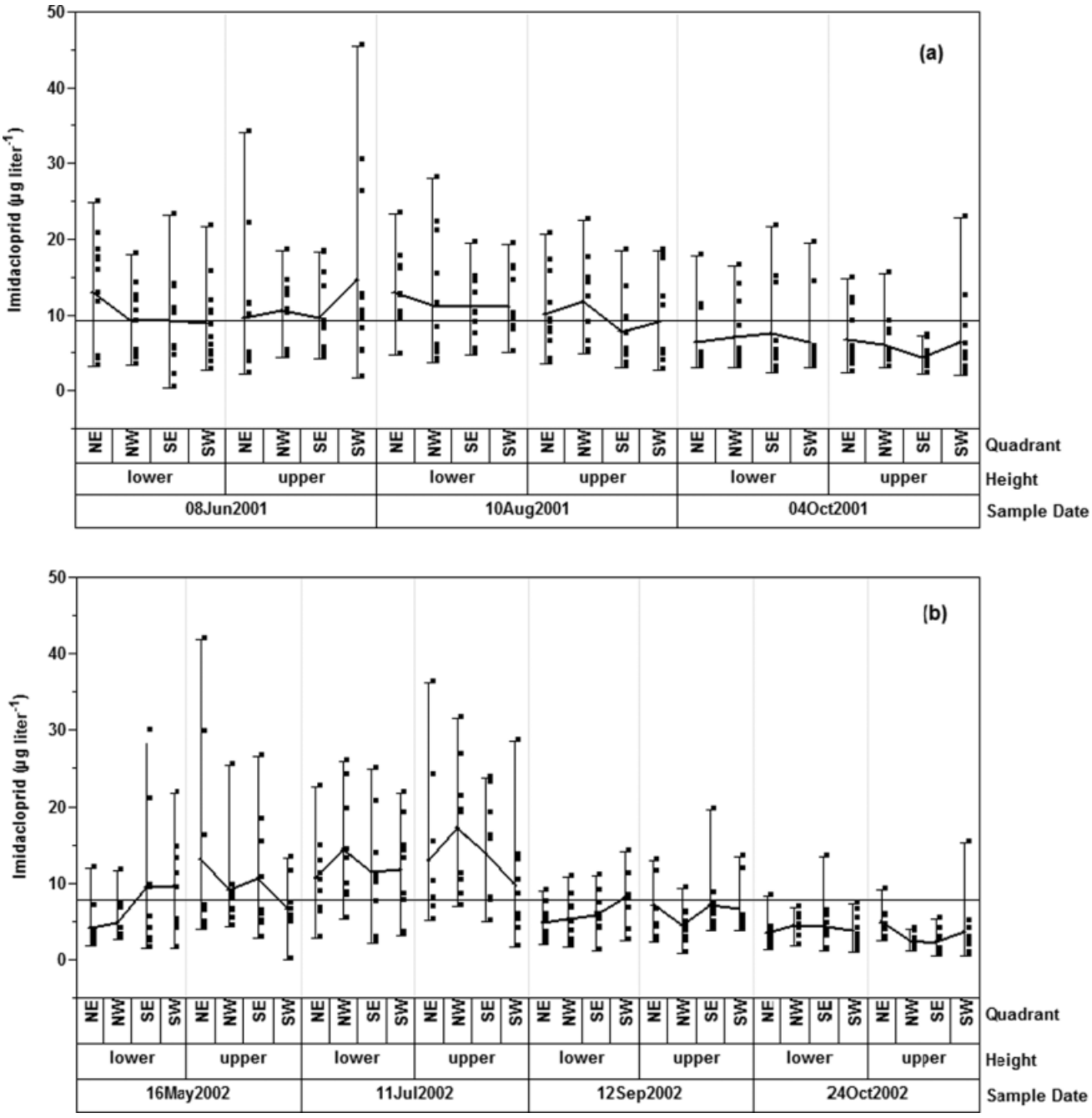

Figure 3. (a) Within-tree spatial patterns of imidacloprid titers in 2001 oranges. Measurements are presented for three sampling dates in each of four quadrants located in both lower and upper halves of the trees. The vertical spans of points represent the range of measurements on a given date with the means for each date connected by the traversing lines. The horizontal line spanning each figure represents the grand mean across all dates. Sample size for each date was $n = 4$ trees and $n = 3$ branches per quadrant in both lower and upper halves of trees. (b). Same description as for (a) except measurements were made on three trees on each of four dates.

during sampling. Contributing to the influx of young adults into the imidacloprid-treated oranges was the absence of any buffer zones between the treated and untreated trees (two rows of treated trees only). By week 14, however, a divergence in the mean number of adults caught in each treatment had begun, reaching its greatest separation in week 18 (17 August). The large difference in adult densities was consistently sustained through week 25 (5 October) after which treated and untreated densities began to converge (Fig 5b). A repeated measures MANOVA conducted on adult densities between 15 June and 30 November 2001 indicated a highly significant difference ($F_{1,21} = 98.9$; $P < 0.0001$) between treated and untreated oranges.

In contrast to the generally steady comparison observed for GWSS nymphs in oranges, the comparison of nymphal densities in treated and untreated lemons lacked consistency (Fig 6a). Numbers of nymphs in untreated lemons were especially erratic, thus making it difficult to observe any clear treatment effect ($F_{1,12} = 0.76$; $P = 0.40$). However, a very similar pattern to the oranges was observed for GWSS adults in treated and untreated lemons in terms of relative densities and degree of separation

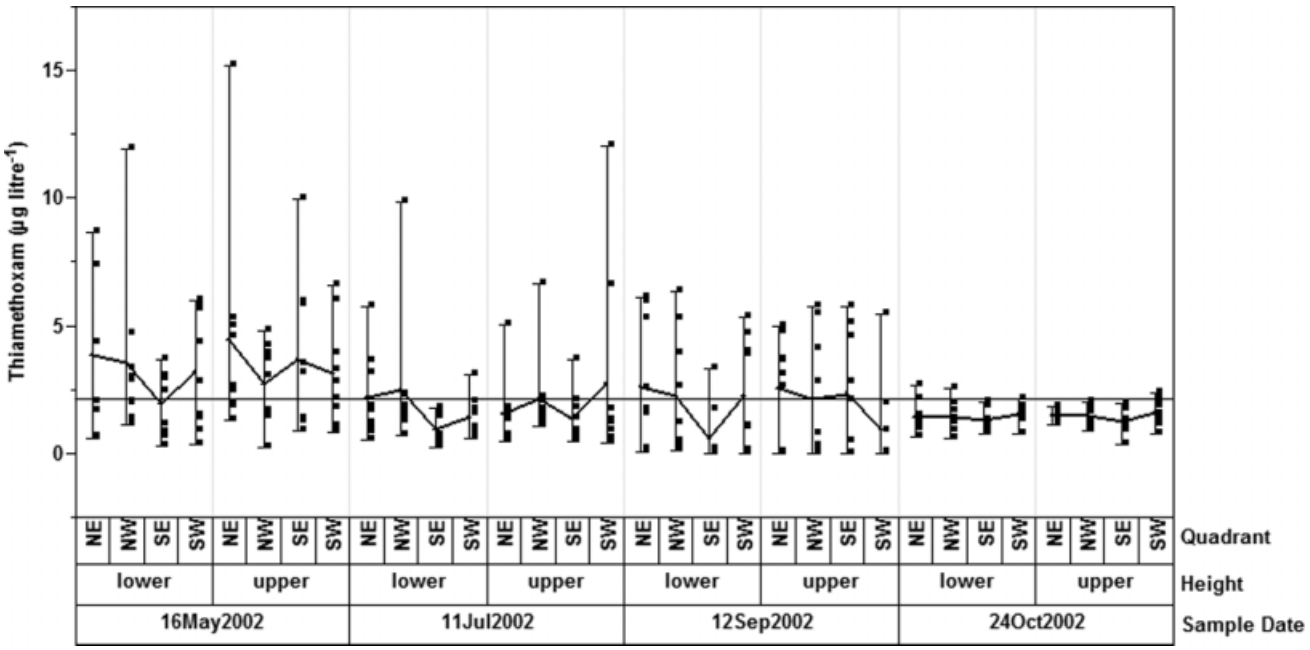

Figure 4. Within-tree spatial patterns of thiamethoxam titers in 2001 oranges. Sample size for each of the four dates was $n = 3$ trees and $n = 3$ branches per quadrant in both lower and upper halves of trees. All other descriptions are the same as for Fig 3.

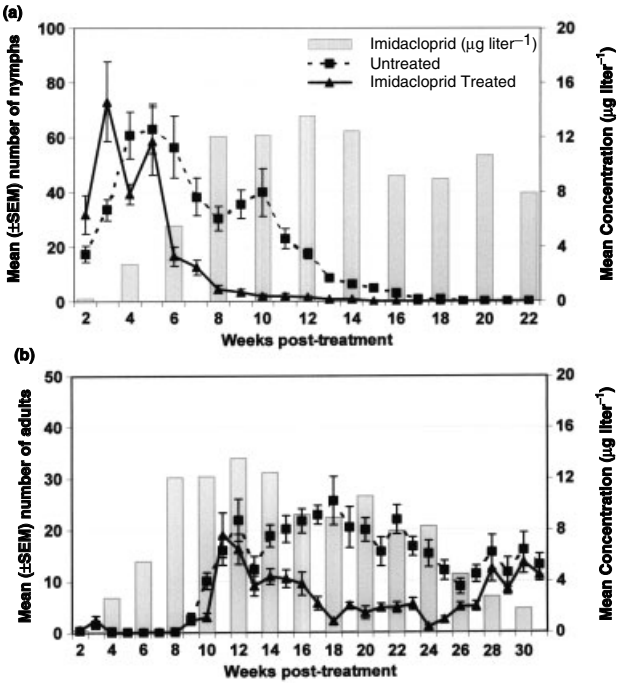

Figure 5. Mean (±SEM) numbers of (a) GWSS nymphs and (b) adults on orange trees treated with imidacloprid compared with untreated trees in 2001. Also presented are mean titers of imidacloprid (ex Fig 1). The *X*-axis extends to only 22 weeks for nymphs whereas 32 weeks are presented for the adults. Note that the *Y*-axis is also different for nymphs and adults. Sample size each week was $n = 12$ trees using a bucket sampler thrusted at five different locations per tree.

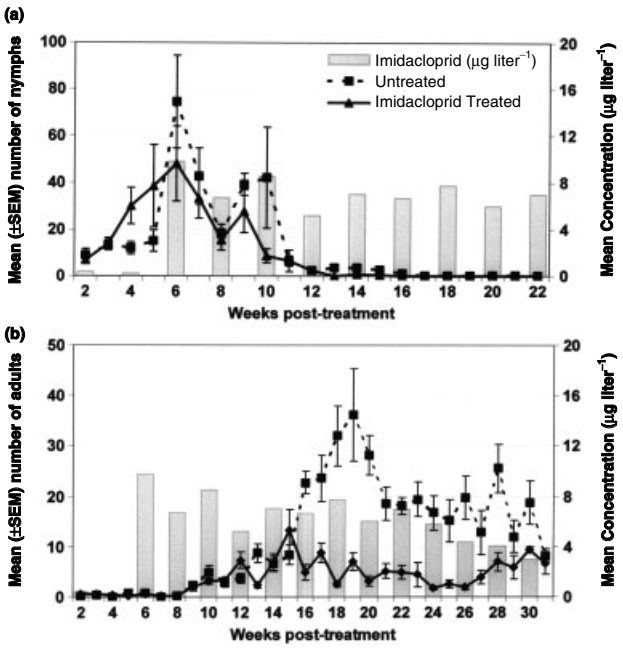

Figure 6. Mean (±SEM) numbers of (a) GWSS nymphs and (b) adults on lemon trees treated with imidacloprid compared to untreated trees in 2002. Also presented are mean titers of imidacloprid for 2002 lemons (ex Fig 1). (See Fig 5 for fuller description of figure symbols).

(Fig 6b). Although mean titers of imidacloprid in lemons remained lower than in oranges most of the season, they exceeded the titers in oranges on the last two dates (weeks 28 and 30) which may have contributed to the more persistent and highly significant ($F_{1,12} = 190$; $P < 0.0001$) difference in adult densities between treated and untreated lemons.

Population densities of GWSS overall were much lower in 2002, possibly contributing to less striking treatment differences than observed in 2001. This was especially true for nymphal densities that peaked at a mean level of only 2.5 (±0.5) on untreated trees. However, a significant treatment effect ($F_{2,21} = 4.7$; $P = 0.02$) was observed among the three treatments

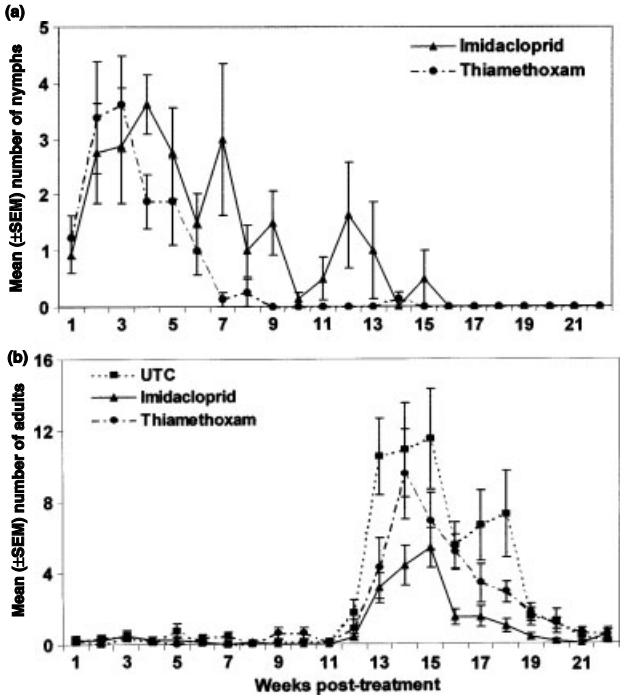

Figure 7. Mean (±SEM) numbers of (a) GWSS nymphs and (b) adults on orange trees treated with thiamethoxam or imidacloprid in 2002. The *X*-axis extends to only 22 weeks for both nymphs and adults; after this time, no more nymphs or adults were captured. Note that the *Y*-axis is also different for nymphs and adults. Sample size each week was $n = 12$ trees using a bucket sampler thrusted at five different locations per tree.

with subsequent pairwise contrasts indicating significantly lower densities ($F_{1,21} = 9.3$; $P = 0.006$) on thiamethoxam-treated than on imidacloprid-treated trees (Fig 7a), but not so for either treatment compared with the untreated control ($F_{1,21} = 2.6$; $P = 0.12$). For adults, a fairly consistent pattern was established early and sustained through much of the evaluation period (Fig 7b). Significant differences ($F_{2,21} = 11.0$; $P = 0.0005$) among the three treatments were observed when comparing adult densities. Contrasts between treatments revealed significantly higher adult densities in untreated trees compared with either thiamethoxam-treated ($F_{1,21} = 6.0$; $P = 0.02$) or imidacloprid-treated trees ($F_{1,21} = 21.9$; $P < 0.0001$). Significantly lower ($F_{1,21} = 5.0$; $P = 0.04$) adult densities occurred in imidacloprid-treated than thiamethoxam treated trees (Fig 7b).

4 DISCUSSION

Prior to the first applications of imidacloprid made in the spring of 2000 against GWSS infestations in Temecula, CA, there had been very limited experience with imidacloprid in citrus or against GWSS in any crop. Expectations were high, none the less, based on knowledge of the superior performance of imidacloprid against sucking pests in various other crop settings.[6–9] Thus, what was initially perceived as poor performance in Temecula citrus 4–6 weeks post-application can now be re-evaluated in the context of the temporal and spatial patterns of imidacloprid uptake and distribution in citrus that we have determined in the present study. In two of three evaluations, the first spike of imidacloprid was detected at six weeks, and in the third evaluation at eight weeks post-application. The rise in imidacloprid titers corresponded nicely to a decline in nymphal densities in 2001 oranges, but was not as apparent in lemons or during the evaluation the following year when nymphal densities were so low. The temporal pattern of imidacloprid titers remained fairly constant for as long as 14–18 weeks post-application before gradually declining. Even at 24 weeks post-application, imidacloprid titers in lemons and oranges in 2001 were still at >60% of their respective peak titers. These titers had a prolonged impact on adult densities, persisting almost to the end of the evaluation in lemons and oranges in 2001. In comparison, treatment differences in 2002 were not as pronounced as they were in the previous year, possibly due to diminished GWSS populations overall, especially nymphal densities. However, recruitment of adults from surrounding orchards during weeks 12–15 increased densities in all three treatments, but at levels that were significantly lower for both thiamethoxam and imidacloprid-treated trees than for untreated trees. The effects of thiamethoxam on GWSS adult densities were apparent even though mean titers at 12–15 weeks post-application were substantially reduced from their peak levels. During the same interval, imidacloprid titers were still moderately high, on the basis of ELISA results that were corroborated by correspondingly lower densities of GWSS adults relative to thiamethoxam-treated or untreated trees.

The combined approach of measuring imidacloprid and thiamethoxam titers directly in xylem fluid samples and indirectly recording their activity in citrus by measuring their impact on GWSS populations enhanced our understanding of their pest management potential. For example, at the time that GWSS adults began to emerge in mid-June and rapidly increase on both treated and untreated trees, questions about the presence and/or consistency of imidacloprid and thiamethoxam titers would certainly be raised without the corroborating direct measurements of each compound. In 2001 oranges, imidacloprid titers were at their peak at the time that GWSS adult densities increased sharply. It is clear that protection by imidacloprid was not sporadic or spatially uneven, but rather was confronted by a population phenomenon where mass emergence of adults coupled with heightened flight activity simply overwhelmed both treated and untreated trees in the orchard. After a few weeks, GWSS adult numbers began to decline and remained consistently and significantly lower than untreated oranges and lemons. Similarly, a rapid increase in nymphal densities occurred in both treated and untreated oranges in 2001, much as they were observed to have done in Temecula in 2000. This phenomenon of increasing GWSS densities weeks

after treatment would continue to raise doubts in the absence of the results presented herein that directly measured the relatively slow uptake and distribution of imidacloprid in mature citrus (6–8 m height). Even after mean titers of imidacloprid increased, GWSS nymphs and adults may have continued to survive for considerable periods by not feeding or by feeding selectively. The antifeedant effects of imidacloprid on other herbivores belonging to Hemiptera (Sternorrhyncha) are well established,[10,11] and indirect evidence for its occurrence in this case came from observations of several nymphs collected from imidacloprid-treated trees that had severely reduced abdomens relative to their heads and thoraxes. Such insects appeared to have been starving, as they did not show other signs of intoxication. Although mean titers were generally uniform in all parts of the trees, the range of titers within xylem fluid samples was sufficiently large that highly mobile GWSS nymphs and adults may have survived for prolonged periods by selectively feeding on branches with imidacloprid titers at the low end of the range. Moreover, the significant differences in mean titers among trees may also have contributed to nymphal survivorship, with nymphs able to move from abutting branches of one tree to another. However, mean titers of imidacloprid exceeded 10 μg liter^{-1} at least once during the monitoring for all but one of the seven trees sampled intensively in 2001 and 2002.

Variability in the application through the irrigation system and/or the rate of uptake could have accounted for the significant variation observed among trees. The same phenomenon could be true for trees treated with thiamethoxam that also showed significant variation among trees, but non-significant variation within trees save for a single quadrant. The finding of faster uptake of thiamethoxam in xylem fluid samples is supported not only by the significantly quicker decline in nymphal densities observed in thiamethoxam-treated trees in 2002 but also by the fact that thiamethoxam is more soluble in water (4.1 g liter^{-1}) than imidacloprid (0.51 g liter^{-1}), and therefore more available in the soil for root uptake and translocation.

In view of the different activity profiles between imidacloprid and thiamethoxam in mature citrus trees, the question of which material to apply may arise under different pest infestation circumstances. The wide range of crops on which both materials are used mandates that extending the current results to practical applications be limited to citrus, and then only to large, mature trees such as the ones used in this study. More extensive testing in annual and perennial crops is required to understand the uptake and activity profiles of both insecticides. In mature citrus, the considerable time required for imidacloprid to reach peak titers did not appear to be a disadvantage with respect to the level of impact it had on GWSS nymphs. The rather synchronous occurrence of the spring generation of GWSS nymphs in southern California citrus and the coincident appearance of the first nymphs with our experimental applications insured a substantial period of overlap, despite the prolonged uptake period. Greater understanding of the phenology of GWSS in citrus may provide better guidelines for when to apply imidacloprid in order to account for the lag period between application and full systemic distribution. Under the particular conditions of this study, a mid-March application of imidacloprid may have provided the early start needed to reach peak titers at a time when most of the spring cohort of nymphs was still early instars. This would have ensured a longer period of exposure to younger and presumably less tolerant nymphs, and perhaps could have further reduced the number developing to adults. In contrast, it may have been better to withhold application of thiamethoxam to a later time when most of the spring generation of nymphs would still be present and feeding. The rapid uptake and distribution throughout the large trees and occurrence of peak titers much higher than those seen for imidacloprid could be very effective in reducing the spring generation of GWSS nymphs before they develop to adults.

The availability of commercial ELISA kits for quantification of imidacloprid and thiamethoxam in citrus xylem fluid provided a highly sensitive yet relatively inexpensive method of measuring concentrations of each chemical that occurred systemically within citrus trees. Large numbers of low-volume samples could be processed rapidly to determine titers of either compound. One potential disadvantage of quantifying imidacloprid by ELISA techniques is that cross-reactivity occurs with some of the plant metabolites of imidacloprid. By sampling only xylem fluid that is >95% water and likely devoid of metabolizing enzymes, we assumed that positive detection represented the parent compound imidacloprid, although verification by analytical tests will be necessary. The real advantage of examining the impact of each compound on a xylophagous insect such as GWSS was that xylem fluid could be collected exclusively[12] using the pressure cylinder, then analyzed without further refinement to determine the concentration of either imidacloprid or thiamethoxam. By superimposing changing GWSS densities on corresponding titers of imidacloprid, it was possible to speculate on certain quantitative relationships between imidacloprid titers and their impact on GWSS. For example, a significant decline in nymphal densities in 2001 oranges at six weeks post-treatment occurred at a time when mean imidacloprid titers surpassed 5 μg liter^{-1}. The beginning of the decline in nymphal densities in lemons also occurred at six weeks post-treatment, at which time mean imidacloprid titers had increased to almost 10 μg liter^{-1}, but the possible connection between the two events was obscured by a concomitant decline through week 8 in untreated lemons. Further field testing will be required to determine whether unambiguous declines in GWSS or other pest densities can be related to certain threshold titers of imidacloprid or thiamethoxam in plants. Information of

this type could be quite useful for rapid and efficient monitoring of effective doses of systemic insecticides and for better approximating the activity window of a given insecticide treatment.

Decision-making in pest management has traditionally relied upon field efficacy data that has accumulated over many different trial circumstances and come to represent a particular activity profile for any given insecticide. The credibility of any particular profile will depend not only on the quality of field evaluations, but on the breadth of circumstances under which these evaluations have been made. The potential problem with the indirect approach, ie relying upon differences between treated and untreated populations to describe the activity profile of an insecticide, is that population densities or dynamics are rarely identical across trials, and therefore interpretation of efficacy or activity can only be made on a trial-by-trial basis. Hence, the necessity for a range of trial circumstances in order to develop a consensus activity profile for a given insecticide. The advantage of directly measuring insecticide concentrations on or within a plant is that evaluation criteria do not necessarily depend on measuring the impact on target populations, but instead rely on identifying temporal and spatial profiles of insecticides on or within plants. If these direct measurements can be tied to pest densities in the field or possibly to laboratory bioassay data that provide information on effective doses, then perhaps pest management decisions could be refined based on more direct and accurate measurements of what an insecticide is doing in or on a plant.

ACKNOWLEDGEMENT

The assistance of Mac Learned with chemical applications and Greg Ballmer with sampling is greatly appreciated.

REFERENCES

1 Sorensen JT and Gill RJ, A range extension of *Homalodisca coagulata* (Say) (Hemiptera: Clypeorrhyncha: Cicadellidae) to southern California. *Pan-Pac Entomol* **72**:160–161 (1996).
2 Purcell AH, Saunders SR, Hendson M, Grebus ME and Henry MJ, Causal role of *Xylella fastidiosa* in oleander leaf scorch disease. *Phytopathology* **89**:53–58 (1999).
3 Blua MJ, Phillips PA and Redak RA, A new sharpshooter threatens both crops and ornamentals. *Calif Agric* **53**:22–25 (1999).
4 Purcell AH and Saunders SR, Glassy winged sharpshooters expected to increase plant disease. *Calif Agric* **53**:26–27 (1999).
5 Perring TM, Farrar CA and Blua MJ, Proximity to citrus influences Pierce's disease in Temecula Valley vineyards. *Calif Agric* **55**:13–18 (2001).
6 Palumbo JC, Mullis CH and Reyes F, Efficacy of imidacloprid on whitefly in fall cantaloupes. *Arth Man Tests* **20**:82 (1995).
7 Bi JL, Toscano NC and Ballmer GR, Greenhouse and field evaluation of six novel insecticides against the greenhouse whitefly *Trialeurodes vaporariorum* on strawberries. *Crop Prot* **21**:49–55 (2002).
8 Bi JL, Toscano NC and Ballmer GR, Field evaluation of novel chloronicotinyls and insect growth regulators against the greenhouse whitefly on strawberry. *Hortscience* **37**:914–918 (2002).
9 Young LC, The efficacy of micro-injected imidacloprid and oxydemeton-methyl on red gum eucalyptus trees (*Eucalyptus camaldulensis*) infested with red gum lerp psyllid (*Glycaspis brimblecombei*). *J Arbor* **28**:144–147 (2002).
10 Nauen R, Hungenberg H, Tollo B, Tietjen K and Elbert A, Antifeedant effect, biological efficacy and high affinity binding of imidacloprid to acetylcholine receptors in *Myzus persicae* and *Myzus nicotianae*. *Pestic Sci* **53**:133–140 (1998).
11 Nauen R, Koob B and Elbert A, Antifeedant effects of sublethal dosages of imidacloprid on *Bemisia tabaci*. *Entomol Exp Appl* **88**:287–293 (1999).
12 Scholander PF, Bradstreet ED, Hammel HT and Hemmingsen EA, Sap pressure in vascular plants. *Science (Washington)* **148**:339–346 (1965).